Geographic variation in the leaf essential oils of *Hesperocyparis* in Arizona, New Mexico, Texas and Mexico

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ABSTRACT

The leaf essential oils were analyzed from *Hesperocyparis* (=*Cupressus*) *arizonica* from Arizona, New Mexico and Texas populations and compared to putative *H. arizonica, H. benthamii* and *H. lindleyi* from Mexico as well as to *H. lusitanica* from the type locality. Clustering revealed six groups: *H. arizonica* (AZ, NM, TX); *H. lindleyi*; an un-named Coahuila group; *H. a*. f. *minor*/f. *glomerata*, *H. benthamii* and *H. lusitanica*. The leaf oils of the *H. arizonica - lindleyi* group are dominated by umbellulone (9.6 - 32.3%), limonene (2.5 - 19.0%), β-phellandrene (4.0 - 18.5%), sabinene (1.1 - 10.7%) with moderate amounts of terpinen-4-ol (4.1 - 9.4%), isoabienol (t - 4.9%) and phyllocladanol (0 - 4.6%). Nezukol was found to be very variable, ranging from 0.5 to 15.2%. The leaf oils of *H. arizonica* from Arizona, Cooke's Range, NM and Big Bend, TX form a distinct cluster and were surprisingly uniform, in spite of the large distances between the Arizona - NM populations and Big Bend, TX. Published on-line www.phytologia.org *Phytologia 98(3): 190-202 (July 6, 2016).* ISSN 030319430.
KEY WORDS: *Hesperocyparis* (=*Cupressus*) *arizonica*, *H. lindleyi, H. benthamii, H. lusitanica,* terpenoids, geographic variation, taxonomy.

In the latest nomenclature of the cypresses, Bartel and Price in Adams et al. (2009) described a new genus, *Hesperocyparis*, for the Western Hemisphere (exclusive of *Xanthocyparis vietnamensis* and *Callitropsis nootkatensis*). Bartel made the new combinations of *Hesperocyparis arizonica* (Greene) Bartel and *H. glabra* (Sudw.) Bartel in addition to the other cypresses the Western Hemisphere .

Analyses using RAPDs fingerprinting (Bartel et al., 2003) showed *H. glabra* to be distinct from *H. arizonica*. Contouring the RAPDs clustering of the populations revealed the geographic disjunction between *H. arizonica* and *H. glabra*. It appears that *H. glabra* is restricted to the Interior Biogeographic Provinces (BP) (Arizonan, which is largely below the Mogollon Rim), while *H. arizonica* is found within the "Sky Islands" of the Madrean BP (Bartel, 1993). The Madrean BP, which occurs throughout much of north-central Mexico, only enters the US in southeastern Arizona and extreme southwestern New Mexico. Wolf (1948), Schoenike et al. (1975), Little (2005), Rehfeldt (1997) and others have all concluded that *H. arizonica* does not range north of Greenlee County nor west of Pima County. Bartel (1993) mapped the distributions of *H. glabra* and *H. arizonica* (in Arizona).

Adams et al. (2010) reported on variation in the leaf oils of *H. arizonica* and *H. glabra* (in Arizona). They found the leaf terpenoids clearly separated these taxa but the study was restricted to populations in Arizona. A preliminary comparison of the leaf oils of *H. arizonica* (actually a cultivated *H. glabra* tree in Waco, TX), *H. benthamii,* a putative *H. lindleyi* from Creel, Chih. and *H. lusitanica* has been previously reported (Adams et al. 1997).

This paper presents the leaf oil compositions and analyses of geographical variation of *H. arizonica* from Arizona, New Mexico and Texas populations as compared to putative *H. arizonica/H. lindleyi* from Mexico. In addition, an updated report on the volatile leaf oil compositions of *H. benthamii*, *H. lindleyi* and *H. lusitanica* are presented.

MATERIALS AND METHODS

Collection site information for samples utilized in this study.

***Hesperocyparis arizonica*, United States:**

BC *Adams 11665-11669*. upper Bear Canyon, 11.8 mi n of Houghton Rd along Catalina Hwy, 32º 21.801' N, 110º 42.765' W, 1695 m, Santa Catalina Mtns., Pima Co., AZ;

CF *Adams 11670-11674*, n side of US191 in dry creek bed, 13 mi. n of Clifton, 33° 08.429' N, 109° 22.537' W, 1636 m, Greenlee Co., AZ;

DG *Adams 11675-11679*, Stronghold Canyon East, 8.5 mi w of US 191, along Ironwood Rd., 31º 55.540' N, 109º 58.007' W, 1501 m, Dragoon Mtns., Cochise Co., AZ;

CR *Ferguson 4028 - 4033* (= Lab acc. *Adams 14767-14772*), north slope of Cooke's Range, n of Cooke's Peak, 32° 34' 32.4" N, 107° 43' 41.2" W, 7345 ft., Luna Co., NM; (Note: Co. corrected from Grant to Luna, digitally, 16 July 2016 by RPA, ed.

BB *Joe Sirotnak ns* 1-5 (= Lab acc. *Adams 14585-14589*), Boot Spring, Chisos Mtns., Big Bend Natl. Park, 29° 14' 30.264" N, 103° 17' 49.4874" W, 6800 ft;

***H. arizonica / H. lindleyi / H. benthamii*, Mexico:**

BN *H. benthamii*, *Adams (with Tom Zanoni) 6879* (bulk collection, 5 trees), 8 km NW of Pachuca, Hidalgo, foliage planate, common with *Abies religiosa,* in El Chico Natl. Park, approx. 10 km from jct. with Mex. 105, 2920 m, ca 20° 09' 06" N, 98° 41' 45" W, ex Google Earth;

CM *Adams 6821-6823*, Creel, Chihuahua, 27° 44' n, 107° 38' w, 2250 m;

C1-C5 *Gonzalez et al. 8345a-e* (= Lab acc. *Adams 14598-14602*), Sierra La Concordia, Cañón de Agua Verde; predio San Marcos del Encino, al S de La Casita, Coahuila, 25° 10' 04" N, 101° 26' 11" W, 2202 m;

G1-G5 *Gonzalez et al. 8350-8354* (= Lab acc. *Adams 14603-14607*), *Cupressus* (*Hesperocyparis*) *arizonica* f. *glomerata* from type locality, Río Jaral, cerca del puente Santa Bárbara sobre la carretera a San Miguel de Cruces, al W de Estación Coyotes, Durango, 24° 00' 44" N, 105° 26' 33" W, 2168 m;

Y1-Y2 *Gonzalez et al. 8191-8192* (= Lab acc. *Adams 14609-14610*), Maicoba, al NE, por la carretera 16 (Hermosillo-Chihuahua), al SW del límite Sonora-Chihuahua, 28° 25' 26" N, 108° 34' 18" W, 1560 m, Yecora, Sonora;

C6 *Gonzalez et al. 8343* (= Lab acc. *Adams 14611*), Sierra la Concordia, s of General Cepeda and n of La Casita, 25° 13' 08" N, 101° 26' 10" W, 2022 m, Coahuila.

L1-L5 *Zamudio 17098, 17099a,b,c,d.* (= Lab acc. *Adams 14885-14889*) cultivated from type locality of *Hesperocyparis lindleyi,* between Angangueo and Tlalpujahua, Michoacan, ca. 19° 39' 45" N, 100° 15' 39" W, 3100 m (Google Earth). Cultivated at Patzcuaro, Michoacan, 19°32' N 101°36' W, 2160 m

M1-M6 *Gonzalez et al. 8390a,b,c,d,e,f.* (= Lab acc. *Adams 14890-14895*) *Cupressus* (*Hesperocyparis*) *arizonica* f. *minor* from type locality, Cruz de Piedra, Durango, 23° 49' 49" N, 105° 15' 22" W, ca. 2270 m.

***H. lusitanica*, Portugal:**

LU *H. lusitanica* (Miller) Bartel, *Adams 7071, 7072, 7073*, *7071* collected from one of the original trees at Busaco (= Bussaco), Portugal at a monastery, sign on tree *7071* read 'Planted 1644', *7071* tree ca 30-40 m tall, 1m DBH, 349 yr old (in 2 Feb 1993), 540 m; *7072* and *7073* from younger trees (8m tall x 12 cm

DBH (progeny) planted? or naturally? established from the original trees within the grove of the 6 old, original trees. This grove of cypress is thought to have been established from seed from Mexico.
All specimens are deposited in the BAYLU and CIIDIR herbaria.

Isolation of Oils - Fresh leaves (200 g) were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (ether trap removed) with nitrogen and the samples stored at -20ºC until analyzed. The extracted leaves were oven dried (100ºC, 48 h) for determination of oil yields.

Chemical Analyses - Oils from 5-15 trees of each of the taxa were analyzed and average values reported. The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1 sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see 5 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software.

Data Analysis - Terpenoids (as per cent total oil) were coded and compared among the species by the Gower metric (1971). Principal coordinate analysis was performed by factoring the associational matrix using the formulation of Gower (1966) and Veldman (1967).

RESULTS AND DISCUSSION

From Table 1, one can see that the volatile leaf oils are dominated by umbellulone (9.6 - 32.3%), limonene (2.5 - 19.0%), β-phellandrene (4.0 - 18.5%) and sabinene (1.1 - 10.7%) with moderate amounts of terpinen-4-ol (4.1 - 9.4%), isoabienol (t - 4.9%), and phyllocladanol (0 - 4.6%). Nezukol was found to be very variable ranging from 0.5 to 15.2% (Table 1).

A minimum spanning network, based on 49 terpenoids, revealed six groups: *H. arizonica* (AZ, NM, TX); *H. lindleyi*; an un-named Coahuila group; *H. a*. f. *minor*/f. *glomerata*; *H. benthamii*, and *H. lusitanica*. In addition, *H. arizonica* from Arizona, Cooke's Range, NM, and Big Bend, TX form a distinct cluster (Fig. 1). Interestingly, one plant from Coahuila (C2, 14599) also joins this cluster. The other five plants from Coahuila (C1, C3, C4, C5, C6) form a very distinct cluster (Fig. 1). The Yecora plants are loosely associated with the Coahuila plants.

Hesperocyparis benthamii, El Chico NP, Hgo. and *H. lusitanica*, Bussaco, Port. loosely cluster indicating theirs oils are quite differentiated from *H. arizonica*, *H. lindleyi* and the other cypresses in this study. The distinct oils of *H. benthamii* and *H. lusitanica* are apparent (Fig. 1, Table 3). It is interesting that to date, the population from which *H. lusitanica* seeds were collected and established at a monastery in Bussaco, Portugal in 1644, has yet to be found in Mexico. Terry, Bartel and Adams (2012), using sequences from seven cp, nrDNA and NEEDLY, found *H. lusitanica* in the *arizonica* clade, but not clearly associated with any other species. It might be noted that *H. lindleyi* was not in the study, nor were any other Mexican cypresses. So it is of interest that the terpenoid data do not place *H. lusitanica* close to any oils in the present study, yet by DNA data, it is clearly nested deep in a clade with the Western Hemisphere cypresses (Terry, Bartel and Adams, 2012), not with the Eastern Hemisphere cypresses (*Cupressus, sensu stricto*). Clearly, Terry, Bartel and Adams (2012), with the utilization of considerable DNA sequencing, showed that many of the cypress species in the *arizonica* clade are scarcely distinct and these taxa might well be treated as conspecific. However, it might be noted that verified H. lindleyi was not included in that study. This seems especially true in the Mexican cypresses that seem to intergrade in their oils.

The question of the validity of *Cupressus lusitanica* Miller, is interesting, in that the naming of cultivated plants as distinct species is controversial, especially if that taxon can not be veritied as growing in nature, without cultivation. Marion Ownbey (1950), in a study of difficult, naturalized hybrids of *Tragopogon*, argued that to recognize a taxon as specifically distinct, it must be a natural group, characterized by: 1. A combination of distinctive morphological features (and/or chemical/ DNA features, *my addition here*); 2. The taxa are reproducing under natural conditions; and 3. There is not free gene exchange between the taxa concerned. One may argue about point 3, as many species do produce hybrids which are fertile and can produce even hybrid swarms by back crosses, yet the species remain clear and distinct in most places where they coexist. Ownbey's second premise: taxa are reproducing under natural conditions, is scarcely the case for *C.(H.) lusitanica* at Bussaco, where they are prized and nurtured since 1644. Thus, if *C. (H.) lusitanica* is not found in Mexico, a case might be made that the name is invalid. An interesting idea, beyond the scope of this study.

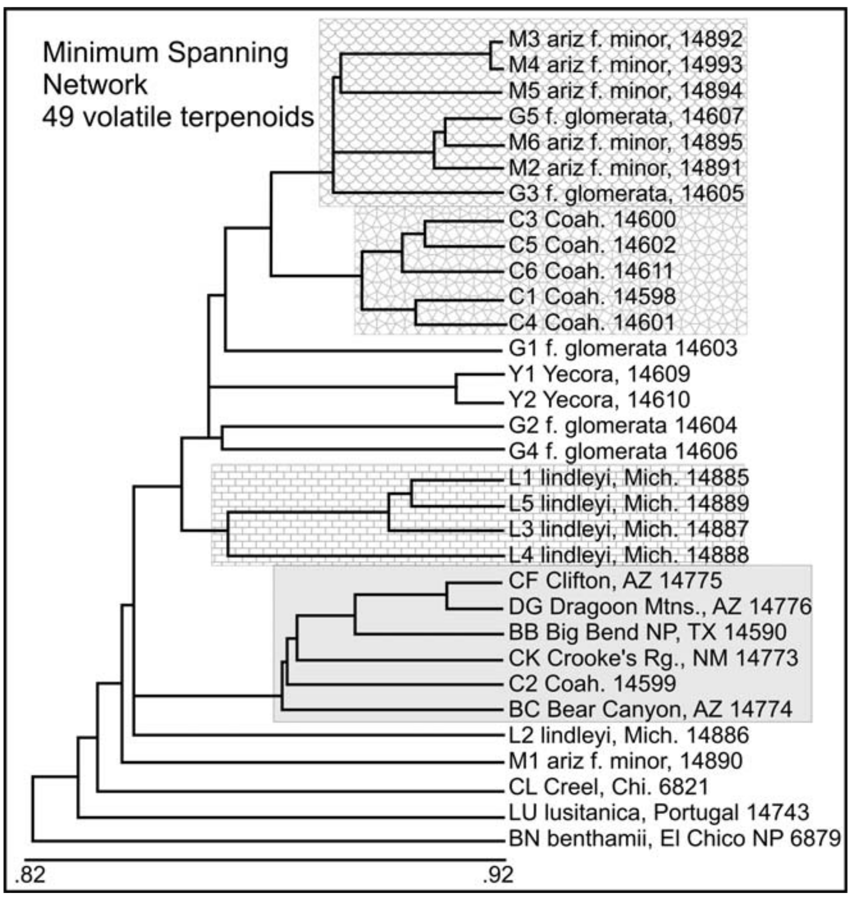

Figure 1. Minimum spanning network based on 49 volatile oil components.

Construction of 2-dimensional minimum spanning network, with distances = [(Sr(max) - Sr(i)] x 100, shows a more details in the magnitude of the links to nearest neighbors (Fig. 2). The six groupings are still present, but the diversity within the groups is now more apparent. The variation in the oils of the *H. arizonica* f. *minor/ glomerata* group is large (Fig. 2) with G1, G2 and G4 having somewhat different oils from the core group (M3, M4, M2, M6, G6). The Coahuila group have five uniform members (C1,C3,C4,C5,C6), but C2, from the same population near Saltillo, has oil that is more like that of *H. arizonica* (Fig. 2). The *lindleyi* group has three very similar members (L1,L3,L5) and two divergent oils in L2 and L4.

Nearly all the groups from Mexico contained chemical polymorphisms. This is shown in table 2 for *H. lindleyi* (L1, L2, L4) and *H. arizonica* f. *minor* (M1, M2, M3, M5). Limonene (and β-phellandrene, not shown) varied from 3.7% to 14.9% (a 4-fold range) as did camphor (0.2 - 11.6%, 58 fold), abietadiene (4.9 - 18.1%, 3.7 fold), and especially nezukol, that varied from 0.0 (absent) to 12.7%. The chemical polymorphisms found in these groups (and others in this study) make it very difficult to utilize terpenoids for systematic purposes. This is unfortunate, as terpenoids can be quite useful in conifers for the analysis of population differentiation and in systematics (see Adams, 2014 for review of use in *Juniperus*).

Again, one sees the divergence of *H. benthamii* and *H. lusitanica* (Fig. 2), whose oils are quite different from any oils in the present study.

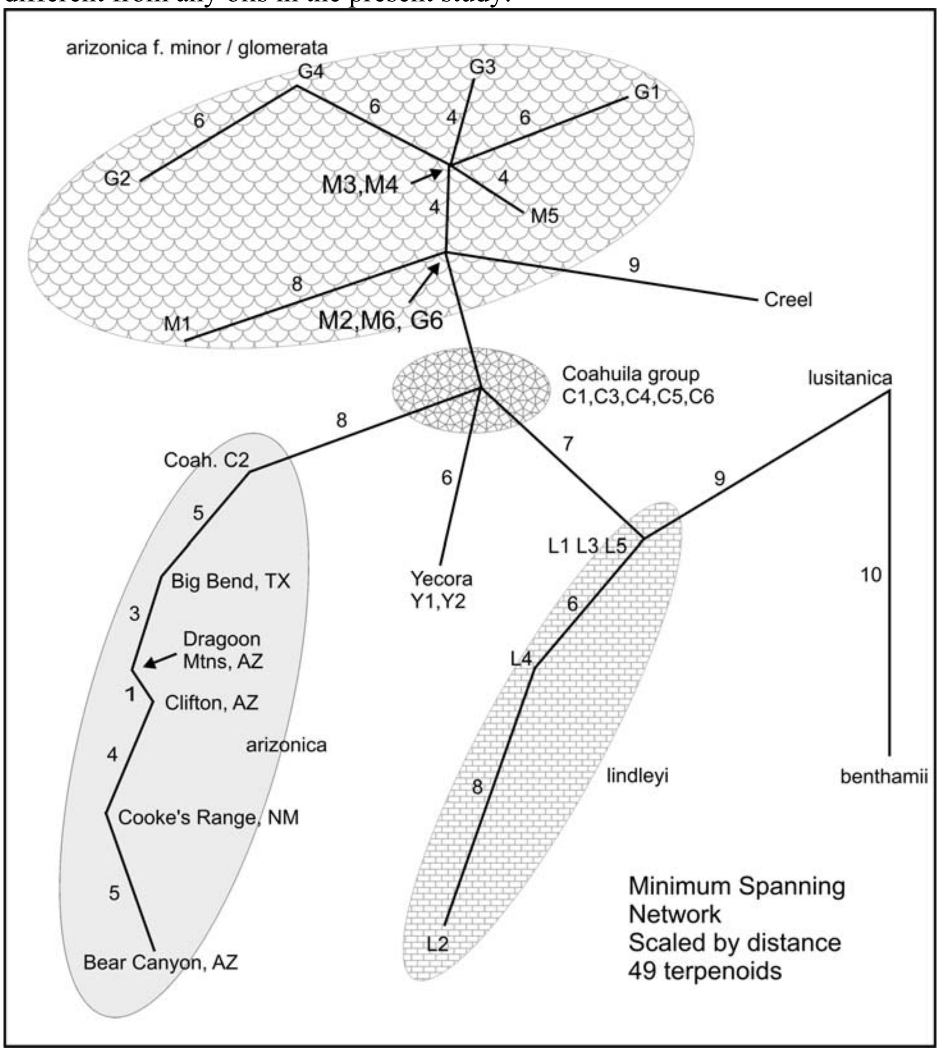

Figure 2. Minimum spanning network with OTUs by distance for *H. arizonica*, *H. lindleyi*, *H. benthamii* and *H. lusitanica* based on 49 leaf terpenoids. The number on a link is distance = [(Sr(max) - Sr(i)] x 100.

To visualize the geographic trends, the minimum spanning network was superimposed on a geographic map (Figure 3). The *H. arizonica* group is clearly defined (dashed ellipse, Fig. 3). It is surprising that the Big Bend population was not very different from the Arizona - New Mexico oils., in contrast to the RAPDs data that showed a clear differentiation by the Big Bend plants from Arizona populations (Bartel et al., 2003). Perhaps the similarities in oil compositions are maintained by selection pressure.

The unusual Coahuila plant (C2) is 0.87 similar to Big Bend, but only 0.84 similar to plants in the same Coahuila population (Fig. 3). The other, typical, Coahuila plant oils are most similar to those of f. *minor/ glomerata* (0.87). The Yecora oil is most similar to the Coahuila oil (0.86, Fig. 3). The Creel oil is not very similar to any of the oils, and joins in the network with a low similarity of 0.83 to the f. *minor*/ *glomerata* group (Fig. 3).

The *H. lindleyi* group's oil is not very similar to other oils, and joins at 0.85 similarity to the Coahuila group (Fig. 3). Notice that *H. lusitanica* (cult, Portugal, exact geographic origin in Mexico is not known) nearest link is to *H. lindleyi* (L4) and that *H. benthamii* links to *H. lusitanica*.

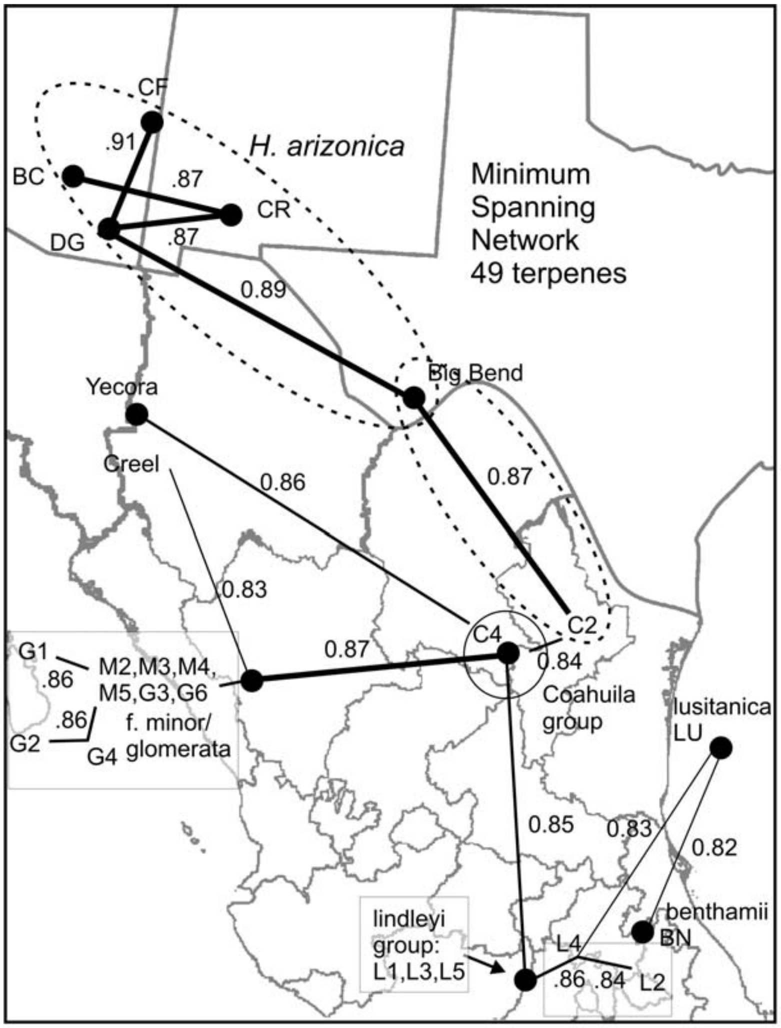

Figure 3. Minimum spanning network with oil similarities next to links.

The similarities among the oils in plants separated by great distances are likely due to a more continuous distribution during the Pleistocene when life zones descended hundreds of meters and discontinuous populations were joined.

The compositions of the leaf volatile oils of *H. arizonica, H. benthamii, H. lindleyi* and *H. lusitanica* are reported in Table 3 so as to correct the previous, erroneous report (Adams et al. 1997).

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Table 1. Leaf essential oil compositions for *H. arizonica* and affiliates. Ariz = composite Dragoon, Bear Canyon and Clifton, AZ populations; CR = Cooke's Rg., NM, BBNP = Big Bend Natl. Pk, Boot Spring; Creel = Creel, Chihuahua; Coah C1 = 14598, C1, Coahuila (typical), Coah C2 = 14599, Coahuila (like BBNP oil); minor = *arizonica* f. *minor*, lindleyi = *H. lindleyi*. Compounds in bold show large differences between the taxa.

KI	compound	CR	Ariz	BBNP	Coah C2	Coah C1	minor	lindleyi	Creel
921	tricyclene	t	0.1	t	t	0.1	t	t	0.6
924	α-thujene	1.0	0.9	0.9	1.0	0.9	1.0	0.6	1.2
932	α-pinene	6.5	3.9	2.9	6.1	3.0	3.4	5.0	4.7
946	camphene	0.1	t	t	t	0.2	t	0.2	0.8
969	**sabinene**	**6.2**	**4.3**	**10.7**	**9.3**	**9.2**	**4.8**	**6.0**	**1.1**
974	β-pinene	0.2	0.2	0.1	0.3	0.1	0.1	0.3	0.1
988	myrcene	1.9	1.7	2.4	2.8	2.0	2.1	2.4	2.8
1002	α-phellandrene	0.1	0.1	0.1	0.3	0.2	0.2	0.1	0.3
1008	**δ-3-carene**	**0.6**	**0.3**	**0.2**	**0.7**	**0.1**	**0.3**	**5.1**	**0.1**
1014	α-terpinene	1.4	1.4	1.8	2.4	1.7	1.9	1.3	1.8
1020	p-cymene	1.7	1.1	0.9	0.5	0.6	1.0	0.6	0.8
1024	**limonene**	**3.4**	**4.2**	**3.7**	**4.4**	**2.5**	**11.0**	**5.7**	**19.0**
1025	**β-phellandrene**	**5.2**	**4.2**	**5.6**	**6.4**	**4.0**	**11.0**	**2.9**	**18.5**
1054	γ-terpinene	2.0	1.8	2.5	3.0	2.2	2.1	1.7	2.6
1065	cis-sabinene hydrate	0.8	0.6	0.9	0.7	1.4	0.8	0.8	t
1086	terpinolene	1.7	1.7	2.0	2.8	2.2	2.1	2.5	2.6
1098	trans-sabinene hydrate	0.7	0.6	0.6	0.5	0.5	0.5	0.3	0.2
1099	linalool	0.3	0.3	0.3	0.2	0.2	1.9	2.2	0.2
1118	cis-p-menth-2-en-1-ol	0.8	0.7	0.9	0.8	0.6	0.7	0.5	0.4
1136	trans-p-menth-2-en-1-ol	0.5	0.5	0.6	0.6	0.3	0.5	0.4	0.3
1141	**camphor**	**0.2**	**0.6**	**0.3**	**0.3**	**2.6**	**0.2**	**2.9**	**1.5**
1145	camphene hydrate	0.6	0.3	1.0	0.2	0.3	0.3	0.3	0.3
1167	**umbellulone**	**18.4**	**19.0**	**20.0**	**19.3**	**32.3**	**27.9**	**17.6**	**9.6**
1174	**terpinen-4-ol**	**7.8**	**5.9**	**9.4**	**7.3**	**5.0**	**5.6**	**5.1**	**4.4**
1179	p-cymen-8-ol	1.0	1.3	0.9	0.3	0.2	0.6	0.3	0.2
1186	α-terpineol	0.7	0.7	0.8	0.7	1.0	1.5	1.2	0.7
1195	cis-piperitol	0.2	0.2	0.2	0.2	0.1	0.2	0.1	t
1205	trans-piperitol	0.3	0.3	0.3	0.3	0.2	0.2	0.3	0.1
1223	citronellol	t	0.1	0.2	t	t	-	-	-
1249	piperitone	0.2	0.2	t	-	-	0.2	0.2	-
1254	linalool acetate	-	0.1	-	-	-	0.1	0.2	-
1287	bornyl acetate	0.1	0.1	t	t	0.3	t	0.3	0.9
1289	thymol	0.1	0.2	-	-	-	0.2	t	-
1299	terpinen-4-yl acetate	0.9	0.9	0.8	0.5	0.2	0.4	0.3	0.4
1346	α-terpinyl acetate	3.0	2.6	2.0	1.6	1.1	1.5	1.6	2.0
1374	α-copaene	-	-	-	-	-	0.1	-	0.5
1417	(E)-caryophyllene	0.1	-	-	0.2	-	0.3	0.1	0.1
1448	**cis-muurola-3,5-diene**	**0.1**	**0.2**	**t**	**0.6**	**-**	**-**	**1.0**	**-**
1452	α-humulene		-		-		0.2	0.1	-
1465	**cis-muurola-4(14),5-diene**	**0.2**	**0.5**	**0.2**	**1.4**	**-**	**-**	**2.5**	**-**
1469	β-acoradiene	-	-	-	-	-	0.2	-	-
1478	γ-muurolene	t	t	t	t	0.2	0.1	-	-
1500	α-muurolene	t	0.1	t	0.4		0.2	-	0.4
1513	γ-cadinene	0.2	0.2	t	t	0.4	0.5	t	0.6
1518	endo-1-bourbonanol	-	t	-	-	1.1	-	-	-
1521	trans-calamenene	0.1	t	0.3	0.1	0.6	t	t	0.8

KI	compound	CR	Ariz	BBNP	Coah C2	Coah C1	minor	lindleyi	Creel
1522	δ-cadinene	0.2	0.8	0.3	0.2	0.6	0.6	0.4	0.9
1537	α-cadinene	-	t	-	-	-	-	-	-
1548	elemol	t	0.2	0.3	0.2	t	0.4	-	0.3
1550	cis-muurol-5-en-4-β-ol	-	-	-	-	-	-	0.3	-
1559	cis-muurol-5-en-4-α-ol	-	-	-	-	-	-	0.5	-
1574	germacrene D-4-ol	0.5	0.9	0.7	-	1.1	0.3	-	0.1
1582	caryophyllene oxide	-	-	-	-	-	0.1	t	-
1600	cedrol	-	-	-	-	-	0.3	-	-
1607	β-oplopenone	0.1	0.2	t	-	t	-	-	0.1
1618	1,10-di-epi-cubenol	t	0.1	-	0.3	-	-	-	0.1
1627	1-epi-cubenol	t	t	0.3	-	0.3	-	-	1.4
1632	**α-acorenol**	**-**	**-**	**-**	**-**	**-**	**1.5**	**-**	**-**
1636	β-acorenol	-	-	-	-	-	0.2	-	-
1638	epi-α-cadinol	0.3	0.6	0.4	0.1	0.5	0.3	0.1	2.2
1638	epi-α-muurolol	0.3	0.6	0.5	0.1	0.5	0.2	0.1	2.1
1644	α-muurolol	0.1	-	0.2	t	0.2	0.1	t	0.8
1652	α-cadinol	0.8	1.6	1.5	0.2	1.0	0.7	0.5	6.2
1688	cis-14-nor-murrol-5-en-4-one	-	-	-	-	-	-	0.2	-
1740	(E)-isoamyl cinnamate	0.1	0.1	-	-	-	-	-	t
1748	(Z)-isoamyl cinnamate	0.2	0.2	t	t	-	-	-	t
1793	(pentenyl cinnamate isomer)	t	t	t	t	0.1	0.2	t	0.5
1887	oplopanonyl acetate	0.9	1.0	0.5	t	t	0.2	-	0.6
1905	isopimara-9(11),15-diene	0.6	0.4	0.2	0.2	0.2	-	t	-
1907	pimara-8(9),15(16)-diene	0.2	0.2	t	-	t	-	-	-
1933	isohibaene	1.1	0.9	0.6	0.7	0.4	-	-	-
1941	sandaracopimara-8(14),15-diene	0.4	0.3	t	t	t	-	-	-
1958	**iso-pimara-8(14),15-diene**	**2.7**	**1.0**	**1.5**	**1.6**	**0.8**	-	t	**-**
1966	**isophyllocladene**	**4.0**	**3.7**	**2.3**	**2.3**	**1.2**	-	t	**t**
1978	manoyl oxide	1.8	2.0	1.5	1.8	0.7	0.5	0.7	t
1987	13-epi-manoyl oxide	0.3	0.5	0.4	0.6	0.2	0.1	-	-
2014	palustradiene	-	-	-	-	-	-	0.4	-
2022	cis-abieta-8,12-diene	-	-	-	-	-	-	0.3	-
2034	kaur-16-ene	0.5	0.4	0.4	0.4	0.2	-	-	-
2055	**abietatriene**	**0.1**	**0.2**	**0.3**	**0.4**	**0.4**	**0.1**	**0.9**	**t**
2087	**abietadiene**	**t**	**0.4**	**2.1**	**t**	**0.1**	**0.1**	**9.2**	**t**
2090	diterpene, 55,41,272,290	0.4	0.4	t	0.2	0.2	-	-	-
2105	**isoabienol**	**0.3**	**0.6**	**3.0**	**4.9**	**2.4**	**0.4**	**1.3**	**t**
2132	**nezukol**	**11.6**	**15.2**	**4.1**	**4.1**	**7.6**	**3.0**	**4.4**	**0.5**
2153	abieta-8(14),13(15)-diene	-	-	-	-	-	-	0.5	-
2209	**phyllocladanol**	**1.5**	**1.3**	**t**	-	-	-	-	-
2184	sandaracopimarinol	-	-	-	-	-	-	0.2	-
2282	sempervirol	0.2	0.3	0.5	1.2	0.6	0.4	0.4	1.1
2314	trans-totarol	0.1	0.2	0.4	0.9	0.5	0.8	0.8	0.6
2331	trans-ferruginol	t	0.1	0.2	0.5	0.2	0.1	0.2	0.3

KI = linear Kovats Index on DB-5 column. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported.

Table 2. Variation in selected terpenoids in *H. lindleyi* (L1, L2, L4) and *H. arizonica* f. *minor* (M1, M2, M3, M5).

KI	compound	L1	L2	L4	M1	M2	M3	M5
1024	limonene				3.7	9.8	14.9	11.4
1099	linalool	1.1	1.7	0.4				
1141	camphor	0.2	11.6	2.0				
1346	α-terpinyl acetate	2.2	0.0	2.7	1.8	1.9	0.5	2.4
1600	cedrol				1.1	0.0	0.0	0.4
1632	α-acorenol				4.5	0.0	2.1	2.1
1978	manoyl oxide				0.4	0.03	0.8	2.4
2087	abietadiene	9.2	4.9	18.1				
2105	isoabienol	0.2	1.6	2.3				
2132	nezukol	4.1	2.7	6.3	0.0	0.0	7.9	12.7

Table 3. Leaf essential oil compositions for *H. arizonica*, *H. benthamii*, *H. lindleyi,* and *H. lusitanica*. ariz = composite oil from Dragoon, Bear Canyon and Clifton, AZ populations; benth = *H. benthamii*, El Chico NP; lusit = *H. lusitanica*, Bussaco, Portugal (ex Mexico); lind = *H. lindleyi*, Angangueo, Michoacan. Major components are in bold. This replaces and corrects the Adams et al. (1977) report of these oils.

KI	compound	lindleyi	arizonica	lusitanica	benthamii
921	tricyclene	t	0.1	t	0.2
924	α-thujene	0.6	0.9	0.3	0.2
932	**α-pinene**	**5.0**	**3.9**	**7.8**	**1.2**
945	α-fenchene	t	-	t	t
946	camphene	0.2	t	t	0.3
969	sabinene	6.0	4.3	6.7	4.3
974	β-pinene	0.3	0.2	0.5	0.5
988	myrcene	2.4	1.7	2.0	2.2
1002	α-phellandrene	0.1	0.1	t	0.1
1008	δ-3-carene	5.1	0.3	3.5	1.2
1014	α-terpinene	1.3	1.4	0.6	1.4
1020	p-cymene	0.6	1.1	0.3	0.6
1024	**limonene**	**5.7**	**4.2**	**1.2**	**2.0**
1025	**β-phellandrene**	**2.9**	**4.2**	**1.2**	**2.0**
1026	1,8-cineole	-	t	0.9	-
1014	(E)-β-ocimene	t	-	0.1	-
1054	γ-terpinene	1.7	1.8	1.1	2.2
1065	cis-sabinene hydrate	0.8	0.6	0.4	t
1086	terpinolene	2.5	1.7	1.1	1.8
1087	2-nonanone	-	t	0.2	-
1098	trans-sabinene hydrate	0.3	0.6	0.5	-
1098	2-nonanol	-	-	0.4	-
1099	linalool	2.2	0.3	-	t
1112	trans-thujone	t	t	t	t
1118	cis-p-menth-2-en-1-ol	0.5	0.7	0.3	0.5
1123	(4-propyl heptane)	-	-	0.4	-
1136	trans-p-menth-2-en-1-ol	0.4	0.5	0.3	0.2
1140	trans-verbenol	0	-	0.2	-
1141	camphor	2.9	0.6	-	t
1145	camphene hydrate	0.3	0.3	0.3	t
1167	**umbellulone**	**17.6**	**19.0**	**2.0**	**5.3**
1174	**terpinen-4-ol**	**5.1**	**5.9**	**3.7**	**3.1**
1178	naphthalene	t	-	0.3	-
1179	p-cymen-8-ol	0.3	1.3	t	-
1186	α-terpineol	1.2	0.7	0.6	0.5
1195	cis-piperitol	0.1	0.2	-	-
1198	shisofuran	-	0.2	-	-
1205	trans-piperitol	0.3	0.3	t	t
1206	verbenone	-	-	t	-
1215	trans-carveol	-	t	-	-
1223	citronellol	-	0.1	t	-
1232	thymol, methyl ether	-	0.1	-	-
1239	carvone	t	t	-	-
1241	carvacrol, methyl ether	-	t	-	-
1249	piperitone	0.2	0.2	-	-
1254	linalool acetate	0.2	0.1	-	-
1287	bornyl acetate	0.3	0.1	t	t
1289	thymol	t	0.2	t	-

KI	compound	lindleyi	ariz	lusit	benth
1299	terpinen-4-yl acetate	0.3	0.9	0.2	-
1315	(2E,4E)-decadienal	-	t	-	-
1319	(2E,4E)-decadienol	-	t	-	-
1346	α-terpinyl acetate	1.6	2.6	0.7	0.1
1396	duvalene acetate	-	-	0.2	-
1407	longifolene	-	-	0.2	-
1417	(E)-caryophyllene	0.1	-	0.5	0.5
1448	cis-muurola-3,5-diene	1.0	0.2	0.6	0.3
1452	α-humulene	0.1	-	0.7	0.3
1465	cis-muurola-4(14),5-diene	2.5	0.5	1.7	1.6
1478	γ-muurolene	-	t	t	-
1479	ar-curcumene	-	-	0.1	-
1500	epi-zonarene	-	t	0.4	1.1
1500	α-muurolene	-	0.1	0.4	-
1513	γ-cadinene	t	0.2	-	t
1514	β-curcumene	-	-	0.1	-
1518	endo-1-bourbonanol	-	t	-	-
1521	trans-calamenene	t	t	0.3	t
1522	δ-cadinene	0.4	0.8	0.2	0.8
1533	10-epi-cubebol	t	-	0.2	t
1537	α-cadinene	-	t	-	-
1548	elemol	-	0.2	-	-
1550	cis-muurola-5-en-4-β-ol	0.3	0.1	0.6	-
1559	cis-muurola-5-en-4-α-ol	0.5	0.2	0.8	t
1565	dodecanoic acid	-	-	-	0.3
1574	germacrene D-4-ol	-	0.9	-	-
1582	caryophyllene oxide	t	-	0.7	0.2
1600	cedrol	-	-	0.6	-
1607	β-oplopenone	-	0.2	-	-
1608	humulene epoxide II	-	t	0.3	0.2
1618	1,10-di-epi-cubenol	-	0.1	0.1	t
1627	1-epi-cubenol	-	t	-	0.2
1632	α-acorenol	-	-	2.1	-
1636	β-acorenol	-	-	0.4	-
1638	epi-α-cadinol	0.1	0.6	0.2	0.2
1638	epi-α-muurolol	0.1	0.6	0.2	0.2
1644	α-muurolol	t	-	t	t
1652	α-cadinol	0.5	1.6	0.9	0.5
1685	germacra-4(15),5,10(14)-trien-1-a	-	-	0.3	-
1688	cis-14-nor-muurol-5-en-4-one	0.2	0.1	-	-
1724	(E)-nuciferol	-	-	0.1	-
1740	(E)-isoamyl cinnamate	-	0.1	0.2	-
1748	(Z)-isoamyl cinnamate	-	0.2	-	-
1793	(pentenyl cinnamate)	t	-	-	-
1887	oplopanonyl acetate	-	1.0	-	-
1905	isopimara-9(11),15-diene	t	0.4	-	-
1907	pimara-8(9),15(16)-diene	-	0.2	-	-
1933	isohibaene	-	0.9	-	-
1941	sandaracopimara-8(14),15-diene	-	0.3	-	-
1958	iso-pimara-8(14),15-diene	t	1.0	0.6	0.7
1959	hexadecanoic acid	-	-	-	0.6
1966	isophyllocladene	t	3.7	-	-

KI	compound	lindleyi	ariz	lusit	benth
1978	manoyl oxide	0.7	2.0	1.5	-
1987	13-epi-manoyl oxide	-	0.5	-	-
2014	palustradiene(abieta-8,13-diene)	0.4	-	1.0	0.5
2022	cis-abieta-1,12-diene	0.3	-	0.6	0.2
2034	kaur-16-ene	-	0.4	-	-
2055	abietatriene	0.9	0.2	2.8	1.8
2087	**abietadiene**	**9.2**	**0.4**	**26.0**	**15.9**
2090	diterpene, 55,41,272,290	-	0.4	-	-
2105	isoabienol	1.3	0.6	1.5	0.2
2132	**nezukol**	**4.4**	**15.2**	**2.4**	**1.2**
2153	abieta-8(14),13(15)-diene	0.5	-	0.9	0.4
2184	sandaracopimarinal	0.2	-	0.6	0.4
2203	diterpene,43,232,275,290	-	-	-	1.0
22 16	diterpene,41,183,141,253,286	-	-	-	0.6
2209	phyllocladanol	-	1.3	-	-
2269	sandaracopimarinol	0.2	-	0.2	0.3
2282	sempervirol	0.4	0.3	2.1	3.9
2300	diterpene,41,107,245,288	-	-	1.3	1.8
2314	**trans-totarol**	**0.8**	**0.2**	**3.8**	**10.1**
2331	trans-ferruginol	0.2	0.1	1.1	2.4
2401	abietol	-	-	t	0.2
2439	diterpene,41,69,301,316	-	-	0.5	1.1

KI = linear Kovats Index on DB-5 column. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported.